



Contents lists available at ScienceDirect

## General and Comparative Endocrinology

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# Leptin levels in free ranging striped mice (*Rhabdomys pumilio*) increase when food decreases: the ecological leptin hypothesis



Carsten Schradin<sup>a,b,c,\*</sup>, Julien Raynaud<sup>d</sup>, Mathilde Arrivé<sup>a,b</sup>, Stéphane Blanc<sup>a,b</sup>

<sup>a</sup> Université de Strasbourg, IPHC-DEPE, 23 rue Becquerel, 67087 Strasbourg, France

<sup>b</sup> CNRS, UMR 7178, 67087 Strasbourg, France

<sup>c</sup> School of Animal, Plant and Environmental Sciences, University of the Witwatersrand, Johannesburg, South Africa

<sup>d</sup> Institute of Evolutionary Biology and Environmental Studies, University of Zurich, Winterthurerstrasse 190, 8057 Zurich, Switzerland

## ARTICLE INFO

### Article history:

Received 22 April 2014

Revised 13 June 2014

Accepted 21 June 2014

Available online 30 June 2014

### Keywords:

Social flexibility

Intra-specific variation in social organization

Drought

Corcorticosterone

Desert

Alternative reproductive tactic

## ABSTRACT

Leptin is a hormone informing the body about its fat stores, reducing appetite and foraging and as such reducing fattening of individuals. In laboratory rodents, leptin secretion is highly correlated to the amount of adipose tissue. We compared this to the alternative ecological leptin hypothesis, which based on the behavioural effects of leptin predicts that leptin levels are disassociated from adipose tissue when fattening is of evolutionary advantage to survive coming periods of low food availability. Studying a species that has to survive a dry season with low food availability, we tested the ecological leptin hypothesis, predicting low leptin levels when food availability and thus adiposity is high promoting foraging and fattening, but high leptin levels in the seasons of low food availability, reducing energetic costs due to foraging. We measured leptin levels in 154 samples of free living African striped mice (*Rhabdomys pumilio*). Striped mice gain significant body mass during the moist season to survive the following dry season with low food availability. We found a strong seasonal effect, with higher leptin levels in the dry season with low food availability, which was in contrast to the hypothesis deriving from studies on laboratory rodents, but in agreement with ecological leptin hypothesis: leptin levels remained low in the period of high food availability, allowing fattening, but increased during periods of low food availability, possibly suppressing energetically costly foraging in an environment where foraging success would have been very low. Leptin correlated significantly and negatively with testosterone levels, and high testosterone levels in the moist season could explain why leptin levels were low even though food availability was high. However, analysing samples from an experimental laboratory study where testosterone levels were increased via implants found no support for a suppressive role of testosterone on leptin. In sum, our study indicates that in a species with seasonal fattening, leptin levels might be uncoupled from the amount of adipose tissue.

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## 1. Introduction

Leptin is mainly secreted from adipose tissue and is informing the hypothalamus about the state of energy balance (Choi et al., 2008), reducing appetite and increasing energy expenditure during periods of increase adiposity (Diamond, 2003). Furthermore, leptin plays a role in reproduction, especially in females, and females typically have higher leptin levels than males (Casanueva and Dieguez, 1999). The regulation of metabolism, reproduction and feeding behaviour is especially important for free living animals in seasonally changing environments, but most studies were done

on laboratory rodents kept under standardized, non-challenging conditions (Casanueva and Dieguez, 1999; Gautron and Elmquist, 2011).

Few studies measured leptin levels in free ranging animals. Some studies found leptin levels to be positively correlated with body fat in free ranging small mammals indicating that leptin as an adiposity signal might regulate seasonal changes in body mass and foraging (Li and Wang, 2005; Wang et al., 2006; Zhang and Wang, 2007), which is in agreement with studies on laboratory rodents (Casanueva and Dieguez, 1999; Gautron and Elmquist, 2011). For example a decline in body mass in winter in Brandt's voles (*Microtus brandti*) was associated with a decrease in leptin levels (Li and Wang, 2005). A decline of leptin levels in winter was also found in Mongolian gerbils (*Meriones unguiculatus*), but at the same time their body mass increased, which would have

\* Corresponding author at: CNRS, UMR 7178, 67087 Strasbourg, France. Fax: +33 (0)3 88 10 69 44.

E-mail address: [carsten.schradin@iphc.cnrs.fr](mailto:carsten.schradin@iphc.cnrs.fr) (C. Schradin).

predicted an increase, not a decrease of leptin levels (Zhang and Wang, 2007). Leptin levels drop significantly in fasting golden spiny mice (*Acomys russatus*), but fail to increase when they refeed and gain fat mass (Gutman et al., 2006). It is possible that the dissociation between leptin and adipose tissue in spiny mice allows them to continue eating and to accumulate fat when food is plentiful, while avoiding compensatory increased energy expenditure during foraging when foraging does not lead to a net energy gain. In bats, which increase adipose tissue before hibernating, leptin levels can be dissociated from adipose tissue, such that the appetite reducing signal of leptin might be absent during the fattening period (Kronfeld-Schor et al., 2000). Also in migratory birds, which often have high fat stores, no correlation between adipose tissue and leptin was found (Gogga et al., 2013). In sum, several field studies were opposing the general assumption that leptin is positively correlated with adipose tissue (Table 1). This lead us to formulate the ecological leptin hypothesis: leptin levels can be dissociated from the amount of adipose tissue, when seasonal fattening is of advantage in a given environment characterized by seasons of low food availability. This hypothesis predicts for species living in environments with seasonal decreases in food availability low leptin levels when food availability and thus adiposity is high to enable surviving the coming season of low food availability, but that leptin levels are high in seasons with low food availability, to reduce ineffective foraging effort. This is in contrast to studies on laboratory rodents, where typically leptin would be high in adipose animals.

Leptin might play a role in the seasonal regulation of body mass and foraging behaviour. Other hormones that are modulating metabolism and reproduction are known to change seasonally, especially thyroxin (Plasqui et al., 2003), glucocorticoids and androgens (Reeder and Kramer, 2005; Schradin, 2008b; Wingfield, 2008). These hormones might influence leptin level or might be influenced by leptin levels. For example, leptin inhibits testosterone production in the testis of mice and sheep (Herrid et al., 2008). Leydig cells in the testis of rodents have leptin receptors (Caprio et al., 1999) and leptin is known to inhibit testosterone secretion from the testis of rats (Tena-Sempere et al., 1999). Leptin is also regulated by glucocorticoids and both types of hormones might work together to regulate the energy balance of an organism (Casanueva and Dieguez, 1999). Seasonal decreases of testosterone and corticosterone might enable organisms to reduce their energy spending in periods of low food availability (Reeder and Kramer, 2005; Schradin, 2008b). In sum, metabolic hormones change seasonally as food (=energy) availability changes, and leptin might correlate with seasonally changing hormones such as testosterone and corticosterone.

The striped mouse (*Rhabdomys pumilio*) from the Succulent Karoo semi-desert of South Africa is an interesting species to study seasonal changes of leptin levels in a free ranging animal. In the Succulent Karoo, breeding takes part during a short 3 months period in spring, which is the season of highest food abundance after rain in winter (Schradin and Pillay, 2005a, 2006). After spring, a long hot dry season occurs (December to April) which is characterized by low food abundance (Schradin and Pillay, 2005a, 2006). Mice gain significant weight in spring increasing adipose tissue, and they loose on average 12% of body weight during the following dry season (Schradin and Pillay, 2005a), when they get into allostatic overload type I (energy intake via food < energy expenditure (McEwen and Wingfield, 2003)). The reduction of food during the dry season and thus the resulting allostatic overload is unpredictable, as rainfall might vary between 0 and 151 mm from January to April (average of  $60 \pm 46$  mm, data from 10 years at our field site), and accordingly mortality during the dry season varies between 70% and 99% (Schradin et al., 2012). To be well adapted, striped mice in this habitat must increase body mass during the spring without stopping at a specific body mass, to increase their probability to survive through the coming dry season. During the dry season, food availability is low and mice mainly feed on succulent shrubs of the species *Zygophyllum retrofractum*, which provides food of high quantity but due to its toxins of low quality, making them unpalatable for livestock (information from the local farmers). Thus, in the dry season striped mice rely on low quality food which is easily available and does not need high foraging activity to be exploited. Therefore, during the dry season activity (including foraging) should be decreased to reduce energy expenditure. If leptin works as a signal to reduce appetite and feeding behaviour including foraging, in striped mice leptin levels should be low during the period of high food availability and high during the period of low food availability. This would mean that leptin levels should be low when a lot of adipose tissue is present (when mice are fat in spring) and high when mice are thin and little adipose tissue is present in the dry season. This would be in opposite to the well-known positive correlation between adipose tissue and leptin levels described from laboratory mice (Casanueva and Dieguez, 1999; Gautron and Elmquist, 2011).

Individuals often differ in their energetic requirements, such that they might have different optimal levels of metabolism and foraging activity. This might be represented and regulated in hormones associated to metabolism, including leptin. Males striped mice follow three alternative reproductive tactics (ARTs) that differ in their hormone levels and their resting metabolic rate (RMR) (Schradin, 2008a; Schradin et al., 2009): (1) Philopatric males that stay as non-breeders in their natal group, which have low testosterone and prolactin

**Table 1**  
Examples of field studies on non-model organisms that shows a dissociation between adipose tissue and leptin levels in periods when fattening is of ecological advantage, leading to the formulation of the ecological leptin hypothesis.

Study species	Study and results	Reference
Mongolian gerbils, <i>Meriones unguiculatus</i>	Body mass increases in winter, but leptin levels decrease	Zhang and Wang (2007)
Spiny mice, <i>Acomys russatus</i>	Leptin levels fail to increase as spiny mice gain body mass after fasting	Gutman et al. (2006)
Little brown bats, <i>Myotis lucifugus</i>	Adipose tissue increases before hibernation, but leptin levels decreased	Kronfeld-Schor et al. (2000)
Little brown bats, <i>Myotis lucifugus</i>	Body mass increases before migration without an increase of leptin levels	Townsend et al. (2008)
Dunlin, <i>Calidris alpina</i>	No correlation between fat stores and leptin levels during migration	Gogga et al. (2013)
Raccoon dog, <i>Nyctereutes procyonoides</i>	Autumn fattening prior to winter sleep is associated with low, not high leptin levels, while during the period of fat catabolism (winter sleep) leptin levels are high	Nieminen et al. (2002)
Echidna, <i>Tachygllossus aculeatus</i>	Seasonal changes in body mass and leptin levels fail to reveal a positive correlation between adipose tissue and leptin, because leptin levels are low in the pre-hibernating periods of fattening	Sprent et al. (2012)
Striped mouse, <i>Rhabdomys pumilio</i>	Leptin levels during the season of fattening are not higher than during the season of fasting and body mass loss. In males, leptin levels are even higher during fasting than during fattening	Present study

levels, but high corticosterone levels and high RMR. (2) Solitary living roaming males with low prolactin and corticosterone levels, low RMR, but high testosterone levels. (3) Group-living breeding males that immigrated into groups of breeding females unrelated to them. Breeding males have low corticosterone levels but high prolactin and testosterone levels and high RMR. Hormonal differences in steroid levels have been reported for many species with ARTs (Oliveira et al., 2008), but in how far leptin, which plays a major role in metabolism and foraging, might differ between ARTs is unknown. Leptin as an adiposity signal might regulate seasonal changes in body mass and foraging differently in individuals of different tactics. One might expect that tactics that need more energy, as indicated by higher RMR, might have lower leptin levels.

In the current study we measured leptin levels in free ranging striped mice of different tactics and in different seasons. First, we compared leptin levels between males of the three ARTs, predicting lower levels in philopatric and breeding males, as their RMR are higher and thus they should spend more time obtaining energy via foraging, while roaming males might invest less in foraging but more in sneaking copulation opportunities. Second, we compared leptin levels between the moist season with high food availability, when striped mice have a high body mass, with the dry season, when food availability and body mass are low. We compared between the two alternative hypotheses: (i) leptin secretion is positively correlated with adipose tissue, predicting higher leptin levels in the fatter animals in the moist season and in individuals with high body mass, versus (ii) the ecological hypothesis based on the behavioural effects of leptin, predicting low leptin levels in the moist season promoting foraging and fattening, but high leptin levels in the dry season, reducing energetic costs of foraging. Apart from a seasonal influence on leptin levels (2 categories), we also tested directly for a relationship between food availability (measured monthly) and leptin levels. Third, we tested whether leptin levels correlated with other hormones changing seasonally that might influence metabolism, which were corticosterone and testosterone. As we found a significant negative correlation between testosterone and leptin, we also measured leptin levels in serum samples available from an experimental study where testosterone levels had been increased via implants, predicting reduced leptin levels.

## 2. Materials and methods

### 2.1. Study species

Striped mice breed in the austral spring (August/September to November/December) (Schradin and Pillay, 2005a). Females have two or three litters. Most individuals born during the breeding season remain philopatric as young adults (>6 weeks old) in their natal group, where they remain for the duration of the entire dry season (December–April) and the cold wet winter (May–July), reaching independent breeding status the following spring. Typically, males will then disperse and attempt to immigrate into groups of communally breeding females, while females will remain in their group and breed communally. However, males can also breed as solitary roamers, leaving their natal group at the start of spring when they are 8–12 months old (Schradin et al., 2010). Group-living breeding males represent the bourgeois tactic with the highest reproductive success, philopatric males have the lowest reproductive success, while solitary-living roamers have low success when population density is high, but similar success to territorial breeders when population density is low (Schradin and Lindholm, 2011). Roamers only occur during the breeding season. At the start of the dry season, roamers that have survived join family groups of solitary breeding females and their offspring, becoming group-living.

### 2.2. Study area and study period

The study was conducted in Goegap Nature Reserve in South Africa (S 29 41.56, E 18 1.60). Goegap lies within the semi-desert biome of the Succulent Karoo, which is characterized by cold and moist winters followed by spring with high food abundance and hot dry summers. In spring, the landscape is dominated by short-living ephemerals, otherwise by long living succulent shrubs. 148 blood samples were collected from February 2011 until February 2012, and 6 samples from other years (2007, 2008 and 2010) were included for roamers, as few roamers were present during the 2011 breeding season (which is typical, with roamers being the rarest tactic in most years).

### 2.3. Plant surveys

Striped mice mainly feed on different plant species (Schradin and Pillay, 2006), such that measuring availability of food plants gives a good indication of overall food availability. Plant surveys were carried out on the 15th of each month on monitoring plots located within the home-ranges of eight different groups. Each plot covered an area of 2 × 2 m and was sampled using standard protocols (Braun-Blanquet Method (Werger, 1974); also previously employed for other studies on striped mice (Schradin and Pillay, 2006)). In brief, we recorded the number and palatability of each food plant contained within each monitoring plot (Schradin and Pillay, 2006). As a measure of food availability we took the mean of the eight plots for each month we collected blood samples.

### 2.4. Determination of reproductive tactics

Reproductive tactics were determined by a combination of trapping, behavioural observations and radio-tracking, as done previously (Schradin et al., 2009; Schradin and Yuen, 2011). Trapping was done around nesting sites six days per month. Trapped striped mice were weighed, sexed, and permanently marked with ear tags (National Band and Tag Co., USA) and temporarily with hair dye (Inecto Rapido, Pinetown, South Africa) for individual recognition during behavioural observations (Schradin, 2006; Schradin and Pillay, 2004). All solitary-living individuals and 1–4 striped mice from each group were fitted with radio-collars (Holohil, Canada) and were radio-tracked to determine sleeping sites locations (Schradin and Pillay, 2005b, 2006). Carrying a radio-collar does not significantly affect either corticosterone levels or behaviour in striped mice (Schradin, 2008b). Mice that were trapped at a nest as juveniles (body mass <30 g) and trapped there subsequently, were regarded as philopatrics. Large heavy males that left their natal group and immigrated into a group of communally breeding females were considered as breeding males. Males that left their natal group and slept alone were regarded as roaming males. Females that showed signs of breeding (lactating nipples, open vagina and loss of body mass indicating parturition, or high body mass indicating pregnancy), were regarded as breeding females. All individuals that were categorised as breeders in the breeding season were also categorised as breeders in the following dry season.

### 2.5. Blood sampling

Altogether, we collected 154 blood samples. Mice were trapped at their nests during the morning shortly after they became active to control for possible circadian rhythms of hormone secretion. Mice were trapped within the first 45 min after they became active, which changes seasonally due to changes in sunrise, with the earliest sample being taken at 6:30 in November, and the latest at 8:35 in September. Traps were watched from a distance of 10 m

and, as soon as a mouse entered a trap, it was taken out and anaesthetized with di-ethyl ether (Schradin, 2008b). A blood sample of 200–500  $\mu$ l (depending on body mass) was obtained from a sublingual vein, a less harmful method than traditionally used techniques of blood sampling (Heimann et al., 2009). Each sample was taken within 2–3 min to avoid a stress response (Schradin, 2008b). Samples were stored at  $-20^{\circ}\text{C}$  at the research station and transported to Strasbourg on dry ice for processing.

## 2.6. Hormone assays

We used the commercial EIA kit A05176 for mouse/rat leptin from SPIbio France. Serial dilution of two pools of striped mouse serum paralleled the standard curve. Spiking of two pools with the standards S0.1, S0.2, S0.5, S1.0 and S2.0 added yielded  $100.8 \pm 16.6\%$  and  $94.7 \pm 9.6\%$  recovery. Intra-assay coefficient of variation for one pool of striped mouse plasma was 8.1% for five samples. Inter-assay coefficients of variation for two pools of striped mouse plasma were 11.2% and 13.7% (each pool analysed in four assays).

In 118 (of the 154) samples, we also measured corticosterone and testosterone, using commercial kits from IBL Hamburg, which were previously validated for striped mouse serum (Schradin, 2008b). Procedures were as stated in the kit manuals, but due to high corticosterone levels typical of this species, samples were diluted 1:99. All measurements were well within the standard curve of the assay. Intra- and inter-assay variability was determined with pools from striped mice. Intra-assay variability of eight measurements was 8.3% and 12.3%. Inter-assay variability for five measurements was 6.4% and 2.3%.

## 2.7. Experimental increase of testosterone levels

We found a significant and negative correlation between testosterone and leptin levels in our field samples (see Section 3), which indicates that high testosterone levels could suppress leptin secretion. We therefore measured leptin levels in 25 serum samples that were available from another published study (Raynaud and Schradin, 2014) where testosterone levels were increased in philopatric group-living males ( $36.0 \pm 2.4$  days old) using subcutaneous time-release pellet of 3.5 mg testosterone (Innovative Research of America, Sarasota, FL, USA). Of these, 10 samples were from individuals that received empty control pellets, and 15 from individuals that received testosterone implants.

## 2.8. Data analysis

All data are presented as mean + SEM. We used the statistical software R (version 3.0.2 R Development Core Team 2006) to perform all analyses. We used the packages lmerTest and ppcor. Statistical analyses were controlled and the R script purchased from the company youRstat (Zurich, Switzerland: <http://yourstat.ch>). As some individuals were measured more than once, individual ID was included as random factor in each model. Error degrees of freedom (df) were calculated using the Satterthwaite method. Effects were tested using type III (simultaneous) modelling, i.e. in multiple effects models parameters for each independent variable were corrected for all other fixed effects in the model. We tested residuals for normality using the Shapiro–Wilk test, and we had to log transform leptin data to achieve normality. We used linear mixed models (LMMs) and step wise elimination of non-significant terms.

In the first LMM we tested differences in leptin levels between the three alternative male reproductive tactic (categorical factor) during the breeding season, the only season roamers are present (roamers don't occur during the dry season (Schradin et al.,

2009)). Body mass and food availability were included as numeric co-factors into this model.

To test for a seasonal effect on leptin levels, roamers were removed from the analysis, as roamers do not occur during the dry season. This LMM had season, sex and social category (philopatric or breeder) as dependent variables, all of which were categorical. As food availability was highly correlated with season, it was not included into this model, but into a third model, where it replaced season.

We also run a LMM to test the prediction that breeders decreased body mass during the dry season. As philopatrics can still change body mass because of growth, which is impossible in breeders (>8 months old), we ran one extra model for body mass, only considering breeders.

For an influence of corticosterone and testosterone on leptin levels we used partial correlations, which means correlations taking into account the effect of the other hormone. For this we used the non-parametric Spearman rank correlation and thus the original (non-transformed) leptin data. Partial correlations were calculated separately for males and females, which are known to differ significantly for both hormones (Schradin, 2008b).

## 3. Results

### 3.1. Comparison of leptin levels between three male alternative reproductive tactics

Leptin levels did not differ significantly between male tactics ( $F_{43,9} = 1.95$ ,  $p = 0.15$ ; Fig. 1). Food availability ( $F_{43,9} = 1.21$ ,  $p = 0.28$ ) and body mass ( $F_{37,2} = 0.26$ ,  $p = 0.61$ ) also had no significant effect. After step wise deletion, only reproductive tactic remained, but was still non-significant ( $F_{44,3} = 1.74$ ,  $p = 0.19$ ).

### 3.2. Seasonal changes of leptin levels and correlation with food abundance and body mass

Leptin levels were significantly higher during the dry than during the breeding season ( $F_{119,4} = 23.95$ ,  $p < 0.0001$ ; Fig. 1), and the interaction between season and social category was also significant ( $F_{119,0} = 5.01$ ,  $p = 0.03$ ), as the increase was more pronounced in philopatrics than in breeders. The interaction between sex and social category was also significant ( $F_{119,0} = 5.18$ ,  $p = 0.02$ ), as the increase occurred mainly in males, including male breeders

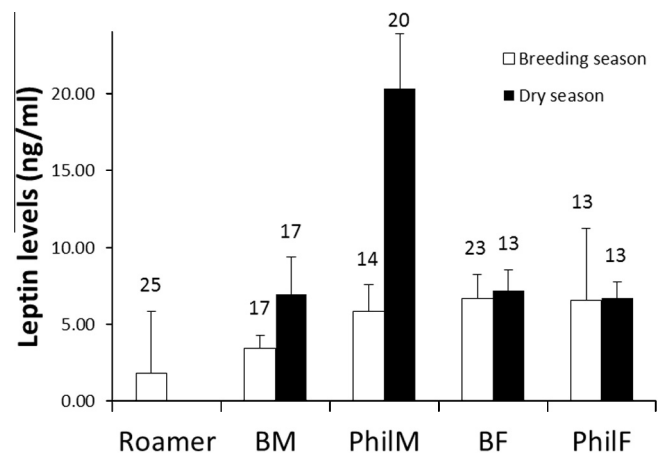


Fig. 1. Mean + SEM leptin levels during the breeding season (white bars) and the dry season (black bars) in different social categories (roamers, which are not present during the dry season; BM: breeding males; PhilM: philopatric males; BF: breeding females; PhilF: philopatric females). Sample sizes are shown above bars. For statistics, see text.



(Fig. 1). Social category ( $F_{119.0} = 2.26$ ,  $p = 0.13$ ), sex ( $F_{106.7} = 0.005$ ,  $p = 0.95$ ), and all other interactions were non-significant.

Leptin levels were significantly lower when more food was available ( $F_{120.2} = 13.18$ ,  $p < 0.001$ ; Fig. 2), and the interaction between food availability and social category was also significant ( $F_{123.9} = 4.89$ ,  $p = 0.03$ ), as the decrease was more pronounced in philopatrics than in breeders. In contrast to the previous model, social category was now significant ( $F_{124.9} = 6.88$ ,  $p = 0.01$ ), but the interaction between sex and social category was not ( $F_{124.9} = 0.89$ ,  $p = 0.34$ ). Sex ( $F_{124.2} = 0.005$ ,  $p = 0.95$ ) and all other interactions were non-significant.

Breeders had significantly lower body mass during the dry season when compared to the moist season ( $55.6 \pm 1.1$  g,  $N = 39$ , versus  $50.4 \pm 1.3$  g,  $N = 33$ ;  $F_{91.28} = 4.13$ ,  $p = 0.04$ ), while sex ( $F_{109.6} = 2.51$ ,  $p = 0.12$ ), and the interaction between sex and season ( $F_{91.30} = 0.18$ ,  $p = 0.67$ ), had no significant influence on body mass. In the model only considering breeders to test for an influence of body mass on leptin levels, season was again significant ( $F_{146.0} = 7.84$ ,  $p = 0.006$ ), as was sex ( $F_{142.4} = 5.09$ ,  $p = 0.03$ ), with female breeder having higher leptin levels. The interaction between body mass and sex was significant ( $F_{140.7} = 5.56$ ,  $p = 0.02$ ), while body mass had no significant influence on leptin levels ( $F_{140.7} = 0.80$ ,  $p = 0.37$ ).

### 3.3. Correlations of leptin levels with corticosterone and testosterone

Leptin correlated negatively and significantly with testosterone both in males (partial  $r_s = -0.30$ ,  $N = 72$ ,  $p = 0.008$ ) and in females (partial  $r_s = -0.40$ ,  $N = 46$ ,  $p = 0.004$ ), while corticosterone did not (males: partial  $r_s = 0.19$ ,  $N = 72$ ,  $p = 0.11$ ; females: partial  $r_s = 0.04$ ,  $N = 46$ ,  $p = 0.80$ ). Corticosterone correlated negatively and significantly with testosterone in males (partial  $r_s = -0.51$ ,  $N = 72$ ,  $p < 0.0001$ ), but not in females (partial  $r_s = -0.14$ ,  $N = 46$ ,  $p = 0.36$ ).

We found testosterone to decrease significantly in the dry season ( $F_{93.0} = 15.40$ ,  $p = 0.0002$ ), to be significantly lower in females than in males ( $F_{93.08} = 10.82$ ,  $p = 0.001$ ; Fig. 3), and to be influenced by the social category ( $F_{93.09} = 4.77$ ,  $p = 0.03$ ), with breeders having higher levels. The interaction between sex and social category was significant ( $F_{93.0} = 11.08$ ,  $p = 0.001$ ), as the decrease was especially pronounced in male breeders, while all other interactions were not significant. However, the Shapiro–Wilk test indicated a significant deviation from normality ( $W = 0.97$ ,  $p = 0.01$ ), even though data were log transformed, and no other transformation solved this problem. Normality was not achieved because of the differences

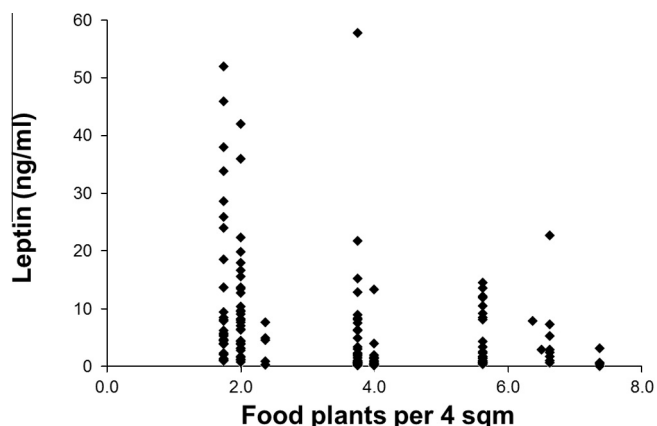


Fig. 2. Correlation ( $F_{120.2} = 13.18$ ,  $r_s = -0.39$ ,  $p < 0.001$ ) between food availability (measured as number of food plant species per  $4 \text{ m}^2$ ) and leptin levels.

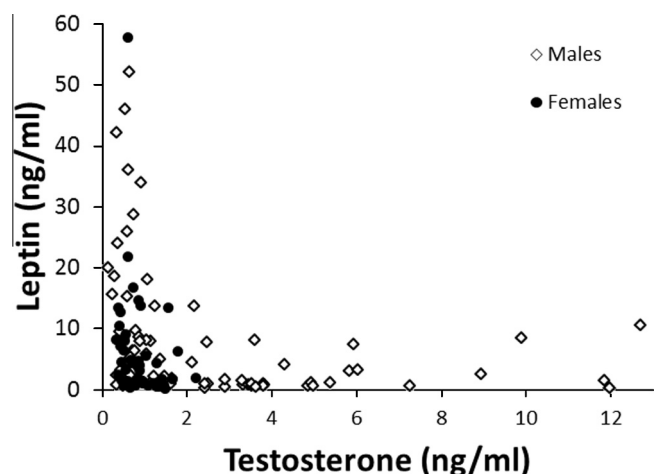


Fig. 3. Correlation between leptin and testosterone levels in female (black dots;  $r_s = -0.40$ ,  $N = 46$ ,  $p = 0.008$ ) and male (white diamonds;  $r_s = -0.30$ ,  $N = 72$ ,  $p = 0.004$ ) striped mice.

between the sexes, which made the distribution too broad. Thus, we also run the LMMs independently for males and for females, reaching normality with log transformed testosterone data. In males, testosterone levels were lower in the dry season ( $F_{50.0} = 9.37$ ,  $p = 0.004$ ), while social category ( $F_{42.0} = 3.02$ ,  $p = 0.09$ ), and the interaction had no effect ( $F_{49.3} = 0.31$ ,  $p = 0.58$ ). In females, testosterone levels were lower in the dry season ( $F_{43.0} = 8.32$ ,  $p = 0.006$ ), while social category ( $F_{43.0} = 1.47$ ,  $p = 0.23$ ), and the interaction had no effect ( $F_{43.0} = 0.03$ ,  $p = 0.87$ ).

### 3.4. Influence of experimentally increased testosterone levels on leptin levels

Individuals with testosterone implants had significantly higher testosterone levels than individuals with placebos ( $18.9 \pm 22.9$  ng/ml versus  $1.5 \pm 1.2$  ng/ml;  $t_{21} = 2.379$ ,  $p = 0.03$ ), but they did not differ in leptin levels ( $5.5 \pm 5.4$  ng/ml versus  $6.7 \pm 7.0$  ng/ml;  $t_{23} = 0.487$ ,  $p = 0.63$ ). There was no correlation between testosterone and leptin levels (Pearson  $r = -0.26$ ,  $N = 23$ ,  $p = 0.23$ ). However, leptin levels correlated significantly and positively with body mass (Pearson  $r = 0.64$ ,  $N = 25$ ,  $p = 0.0005$ ).

## 4. Discussion

Our results are in contrast to the assumption arising from studies on laboratory rodents that leptin levels should increase in periods in which animals gain body mass (Casanueva and Dieguez, 1999; Gautron and Elmquist, 2011). We found low leptin levels in striped mice in the period when they had to gain body fat to survive the coming dry season. In the dry season food availability and body mass declined, but leptin levels did not decline. Instead, leptin levels remained low in females and even increased in males, maybe to reduce appetite and thus energetically expensive and as such ineffective foraging. Thus, our study supports the ecological leptin hypothesis predicting that leptin secretion should be low in periods in which fattening is important to survive coming periods of low food availability.

Previous studies have shown that striped mice following ARTs differ in their steroid hormones (both corticosterone and testosterone), as well as in the peptide hormone prolactin (Schradin, 2008a,b; Schradin et al., 2009). Roamers are expected to spend more energy during roaming and face a trade-off with foraging,

such that they can store less energy in the form of fat. Given the important role of leptin in metabolism and energy balance (Gautron and Elmquist, 2011), we expected solitary roamers to show lower leptin levels than group-living striped mice. However, we did not find any differences between male ARTs, and the leptin levels of solitary roamers were similar to the ones of males following a sociable tactic. Thus, we did not find any indication that leptin would play a role in the regulation of ARTs.

Our study gives support to the notion that leptin levels can be disassociated from the amount of adipose tissue, as indicated already by other field studies on non-model species (Table 1). While we could not directly measure fat mass in our study, several results support this statement: (1) Body mass had no effect in any of the models run. Even when we restricted the data to breeders (to avoid a possible influence of young philopatric adults still growing), which were losing around 10% of body mass from the moist to the dry season, body mass did not influence leptin levels. (2) Food availability was negatively associated with leptin levels, which means when more food was available and mice could store energy as fat, leptin levels were lower, which is in contrast to the hypothesis that food induces increased leptin levels. (3) Leptin levels were not higher during the period of high food availability when the mice were fattening compared to the dry season when mice were losing body mass. The most striking result was that males showed the highest leptin levels in the dry season, when food availability was low. This pattern was especially strong in philopatric males (Fig. 1). Philopatric females can remain in their natal group to breed the next breeding season, but philopatric males will have to disperse and try to immigrate into other groups. To be successful in this, body mass is of crucial importance, as only the heaviest males become group-living breeding males (Schradin et al., 2009) which have the highest fitness (Schradin and Lindholm, 2011). Thus, breeding males have to maintain a high body mass even in the dry season, and philopatric males are the ones that need to avoid body mass loss/put on most weight to enhance their fitness. Whether this could explain the sex differences in seasonal changes of leptin secretion remains to be tested. In sum, we found no indication that leptin levels are positively associated with factors that are correlated with adipose tissue (body mass, food, season of high food availability).

Leptin informs the hypothalamus about the fat stores in the body, down-regulating appetite and feeding behaviour when fat stores are full (Casanueva and Dieguez, 1999; Friedman and Halaas, 1998). Thus, from an ultimate and ecological point of view, whether or not leptin shall be released to reduce food uptake depends on whether or not further fattening is beneficial. Our ecological hypothesis states that leptin levels should not increase with increasing adipose tissue if further fattening is of ecological relevance and thus of evolutionary benefit. This is the case in bats fattening for hibernation (Kronfeld-Schor et al., 2000) and in birds fattening for migration (Gogga et al., 2013). Our study is the first one which finds support that this is also the case in small mammals surviving drought periods characterized by low food availability. This brings one new aspect into this scenario, which are leptin levels in periods of decreasing adipose tissue. In hibernating bats and in migrating birds, behaviour is mainly fixed, they have little choice than to hibernate/to migrate. However, fasting striped mice during the dry season could be induced to increase foraging activity to decrease body mass loss when their leptin levels decrease. However, in contrast to this proximate prediction based on laboratory studies, we found leptin levels to increase, not to decrease during the dry season. In fact, increasing foraging when no food is available in the environment would not decrease the rate of body mass loss, as no additional highly nutritious food could be found. Instead, the energetic costs of foraging could increase the rate of body mass loss. Leptin has also been reported

to increase spontaneous physical activity in rats (Choi et al., 2008) and to influence metabolism by increasing the sympathetic tone (Geerling et al., 2014; Harris, 1842), both of which can lead to a reduction of body mass. However, in wheel running laboratory mice, this effect was only observed in mice selected for high frequency of wheel running but not in mice from a non-selected control strain (Meek et al., 2012). We would expect that spontaneous activity in striped mice is not increased by leptin, as this would lead to non-adaptive high activity in the dry season. In sum, our data support the ecological leptin hypothesis, that leptin levels can be disassociated from adipose tissue when ecological conditions favour continuous fattening.

Leptin can be secreted from various tissues, but the main organ secreting leptin is white adipose tissue (Galic et al., 2010; Margetic et al., 2002). So the question arises how leptin levels could be disassociated from the amount of adipose tissue. Obviously, in this case adipose tissue does not secrete an amount of leptin that is directly dependent on the amount of adipose tissue, but somehow leptin secretion by adipose tissue must be suppressed. Leptin is known to have a negative effect on testosterone secretion (Herroid et al., 2008; Tena-Sempere et al., 1999), and as hormones often influence each other, this makes a feed-back loop likely in which testosterone might suppress leptin secretion. Leptin levels were negatively correlated with testosterone levels, and high testosterone levels during the breeding season could have suppressed leptin secretion, allowing for fattening. However, our experimental study did not find support for a suppressing effect of testosterone on leptin levels. Instead, both hormones might be regulated independently from each other in a way that optimises behaviour and physiology in an environment with extreme seasonal changes in food availability. Alternatively, our study design might not have mimicked well the situation in nature, as our control males were not fasting, but well fed, and as a consequence their testosterone levels were relatively high and their leptin levels were low (comparable to breeding season levels in the field study). In sum, so far it is unknown how leptin secretion from adipose tissue is suppressed in the field, and future experimental studies on a potential influence of testosterone must include individuals with low basal testosterone levels.

## 5. Conclusions

Laboratory studies demonstrated that leptin is secreted by adipose tissue and informs the brain about the state of the fat stores. However, field studies on non-model organisms show that this information flow can be suppressed if ecological conditions favour fattening. In humans, a species with a long history characterised by famines, fasting and starvation, the ecological conditions favouring fattening also have occurred over many generations and in many populations (Diamond, 2003; Keys et al., 1950) (but see Speakman, 2013 for a different opinion). Insensitivity to leptin levels have been studied as one factor leading to obesity in humans (Myers et al., 2010), but factors that can suppress leptin secretion from adipose tissue might be another proximate pathway. Our study found correlative but not experimental evidence that testosterone might suppress leptin secretion, which will need more future experimental studies with a study design focussing on control individuals with low testosterone and high leptin levels. Our study found one new aspect: leptin secretion might even be up-regulated in periods with decreasing adipose tissue, to avoid increased energy spending by ineffective foraging activities. In sum, field studies on non-model organisms reveal that the regulation of leptin secretion and thus the regulation of appetite, feeding and body mass are more complex and diverse than suggested by studies on laboratory rodents.

## Acknowledgments

We wish to thank the manager and staff of the Goegap Nature Reserve for their support and the Department of Tourism, Environment and Conservation of the Northern Cape for research permits. I. Schoepf and C.H. Yuen contributed significantly to collection of blood samples. Funding was provided by the Swiss National Science Foundation (31003A-135770/1 and IZKOZ3\_143150/1), the University of the Witwatersrand and the University of Zurich. Animal ethics clearance was provided by the University of the Witwatersrand (AEC 2007/10/01 for trapping and 2007/39/04 for blood sampling).

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